공학석사 학위논문

Enhanced Mechanical Properties in Electroactive Hydrogels for 3D-Structural Synthesis

전기장 반응성 하이드로겔의 3D 구조체 제작을 위한 물성 강화 연구

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Abstract

Enhanced Mechanical Properties in Electroactive Hydrogels for 3D-Structural Synthesis

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Hydrogels are a homogeneous mixture of water and crosslinked polymer network, showing characteristics of liquid and solid phases at the same time. Among various hydrogels, electroactive hydrogels are one of smart materials which react to the external stimuli and change their forms or functions. Thanks to nature of hydrogels, electroactive hydrogels have unique features such as softness, stretchability and biocompatibility. When the electroactive hydrogels are synthesized in 3D structure, the unique features of hydrogels and the electroactivity enable the structure to be a bio-robot functioning inside the living body. Unfortunately, existing electroactive

hydrogels have poor mechanical properties to be 3D structurally synthesized. Here I reported enhancement of mechanical properties in electroactive hydrogel, PSPA (poly 3-sulfopropyl acrylate) and PAETMAC (poly 2-acryloyloxyethyl trimethyl ammonium chloride) hydrogels, by using modified hyaluronic acid as a crosslinker. Modified hyaluronic acid resolves the problem come from the fast polymerization initiated by LAP, which makes high elastic modulus and high stretchability incompatible. Mechanically enhanced electroactive hydrogels were 3D printed by DLP 3D printing apparatus, and functioned well under an electric field.

Keywords: hydrogel, electro active hydrogel, 3D structure synthesis, mechanical properties enhancement, natural macromolecules, additional crosslinking

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초 록

하이드로젤은 용매인 물과 가교 결합된 고분자 네트워크의 균일한 혼합물로, 용매인 물의 액상 성질과 틀을 형성하고 있는 고분자 네트워크의 고상 성질을 동시에 나타낸다. 이러한 하이드로젤 중 전기장 반응성 하이드로젤은 스마트 재료 중 하나로, 외부의 자극에 반응하여 재료의 형태가 바뀌거나 다른 기능을 하게 되는 재료이다. 전기장 반응성 하이드로젤은 하이드로젤의 성질인 연성, 신축성, 생체적합성을 가지고 있어, 이러한 성질을 이용하여 3차원 구조체로 제작, 체내에서 활동하는 바이오 로봇으로 사용될 수 있는 능력이 있다. 하지만 현재 존재하는 전기장 반응성 하이드로젤들은 낮은 탄성계수, 인장 능력 등으로 인해 쉽게 변형되고 깨지기 쉬워 3차원 구조체로 제작이 되기에 충분한 물성을 가지지 못하고 있다. 이 논문에서는 전기장 반응성 하이드로젤의 일종인 PSPA, PAETMAC 하이드로젤의 낮은 물성을 새로운 가교 결합 분자로 생체고분자인 히알루론산을 사용함으로써 보완했다. 낮은 물성으로 인해 3차원 제작이 어려웠던 문제를 해결하고, 3차원 인쇄를 위하여 제작된 DLP 인쇄 장비를 이용하여 기계적 성질이 향상된 전기장 반응성 하이드로젤을 실제로 인쇄하였다. 이렇게 제작된 하이드로젤 구조체가 전기장 반응성을 유지하고 있음을 확인하였다.

주요어: 하이드로젤, 전기장 반응성 하이드로젤, 3차원 구조체 제작, 물성 강화, 천연 고분자, 추가 가교결합

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Figure set

1. Introduction

1.1 Hydrogel

Hydrogels are crosslinked polymer networks which can contain water inside the networks by virtue of hydrophilic moieties of specific monomers. The polymer networks and the solvent are homogeneously mixed together, assigning stretchability, flexibility, elasticity and softness to hydrogels. Hydrogels can take advantages of water which they are containing, such as biocompatibility, ion conductivity and viscoelastic behaviors. In this perspective, many hydrogels are used in various fields including bio scaffolds for tissue engineering, drug delivery systems[6], stretchable ionic devices[7], ionic actuators and bio-robotics[8, 9].

Mainly composed of water, hydrogels have little different mechanical properties than other polymers. Generally, hydrogels have low elastic modulus of 10~100 kPa, low stretchability of 1.1~4 times stretched and low toughness of 10~1000 J/m^2 [10]. The weakness of hydrogels limits broad application. Many researchers studied about enhancement of mechanical properties in hydrogels. J.-Y.Sun *et al.* reported highly stretchable and tough polyacrylamide/alginate hydrogel which has 100 kPa of

elastic modulus, 21 times of stretchability and ~9,000 J/m^2 of toughness[4]. P.Lin and his team reported covalently and ionically dual crosslinked hydrogel which have elastic modulus of 5 MPa with 5 times stretchability[2]. They have many mechanism of enhancement in mechanical properties, we can modify hydrogels in the way we want.

Interestingly, some kind of hydrogels show special reaction to external stimulus such as magnetic field, temperature and pH thanks to their various moieties, which are ‘smart’ hydrogels functioning tasks by reacting to external environment[9, 11]. These smart hydrogels have been studied animatedly, for use in field of biomaterials.

1.2 Electroactive hydrogel

One of the oldest functionality of hydrogels is electroactivity, which is bending of the gel of change of swellability in the gel under an electric field. Since T.Tanaka and his team published electro-shrinking of hydrolyzed polyacrylamide hydrogels at 1982[12], electro-active hydrogels which bend toward the electrode when an electric field is applied was reported in 1992[13].

Lately, cargo transport in hydrogel walker which walks under an electric field was reported by C.Yang *et al* [1]. In Yang's research, UV-polymerized electroactive hydrogels have different swellability in top and bottom side caused by UV penetration, forming natural bend when fully swelled. The bent hydrogels acted as a walker by alternative motion between huge bend under an electric field and straightening under opposite direction of an electric field.

The research of C.Yang and his team implies the possibility about the electro-active hydrogels which is that the simple bending movement can be complex functions when the hydrogels are fabricated in 3D structures[14]. For example, hydrogel fishes can swim in water under an AC electric field[9] and hydrogel capsules

can release drugs under an DC electric field. It means that elaborately designed 3D structure of a single hydrogel material can be a robot, especially operate inside a living body without any controllers[15].

For acquisition of 3D structured hydrogels, 3D printing technology has to be introduced to the field of hydrogels. Many material science and engineering researchers has been studying about the technologies of 3D printing of hydrogel by co-working with mechanical engineers[5, 16, 17]. In this research, one of those technologies was used.

1.3 Enhancement of mechanical properties in Electroactive hydrogels

Fabricating hydrogels in 3D structures or elaborate shapes is technically hard because of the inherent weakness of them. As described, most electro-active hydrogels have poor mechanical properties such as elastic modulus, toughness and stretchability. Without any enhancement of mechanical properties in pure electro-active hydrogels, many problems in printing process exist. While a 3D structure is fabricated, early fabricated section deviates from the original coordinate because of gravity and material's softness, and this makes fabrication of elaborate shapes of soft materials harder.

Fortunately, there are a lot of enhancing mechanism in mechanical properties. J.-Y. Sun and his team proposed energy dissipation mechanism. Interpenetrating two networks of polyacrylamide and alginate coexist in the same space, and ionically crosslinked alginate network dissipates energy by zipping up the ionic crosslinkings while covalently crosslinked polyacrylamide network maintains their linkage by elastically stretched[4]. P. Len *et al.* introduces ionic and covalent dual crosslinking using polyacrylic acid single network hydrogel with similar

mechanism[2]. S. Hong and his team proposed addition of nanoclay which provides physical crosslinking and enhances mechanical crosslinking[5].

Unfortunately, these mechanisms need an amount of additives which are obvious to block the electroactive mechanism by being existing. Addition of ionic crosslinker hinders ion mobility caused by an electric field, and addition of nanoclay hinders bending movement itself. In order to solve this problem, the mechanical properties of hydrogels must be enhanced without ruining the electro-activity by other methods.

3D synthesis using a 3D printing apparatus

3D printing of hydrogels is not the whole new technology. M. Gou and colleagues studied bio-inspired detoxification using polyethyleneglycoldiacrylate (PEGDA) hydrogels by 3D printing[3]. They proposed liver cell-like hexagonal PEGDA hydrogel structure synthesized by 3D printer. S. Hong and his colleagues synthesized highly stretchable and tough PEGDA/alginate double networks hydrogel with nanoclay in 3D structure using their own 3D printing apparatus. The described examples are not functional hydrogels, however the technologies of 3D printing of hydrogels are noticeable. They enhanced hydrogels' weakness by several methods and printed in 3D structure using various methods.

In this paper, a noble method is developed to improve the mechanical properties of PSPA (poly 3-sulfopropyl acrylate potassium salt) and PAETMAC (poly 2-(acryloyloxy)ethyl trimethylammonium chloride) electro-active hydrogels by using modified natural biopolymer, methacrylated hyaluronic acid (MA-HA). Hyaluronic acid is one of polysaccharides distributed widely in human epithelial tissues, known to be highly biocompatible, hydrophilic and affordable natural biopolymer. Addition of non-charged macromolecules over 2 million of molecular

weight enables the polymer networks to be tangled more complexly, enhancing mechanical properties such as Young ' s modulus and toughness. Based on the mechanically enhanced electro-active hydrogels, 3D structures of them were designed and fabricated by using 3D printing apparatus.

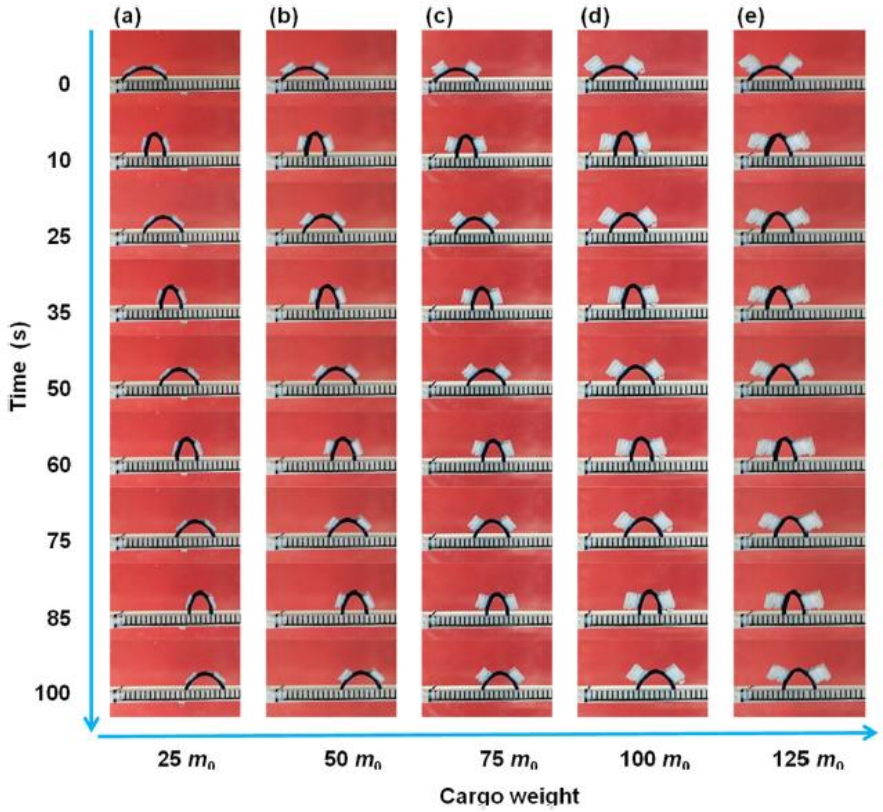

Figure 1.1 Hydrogel walkers reported by C. Yang and his team[1]

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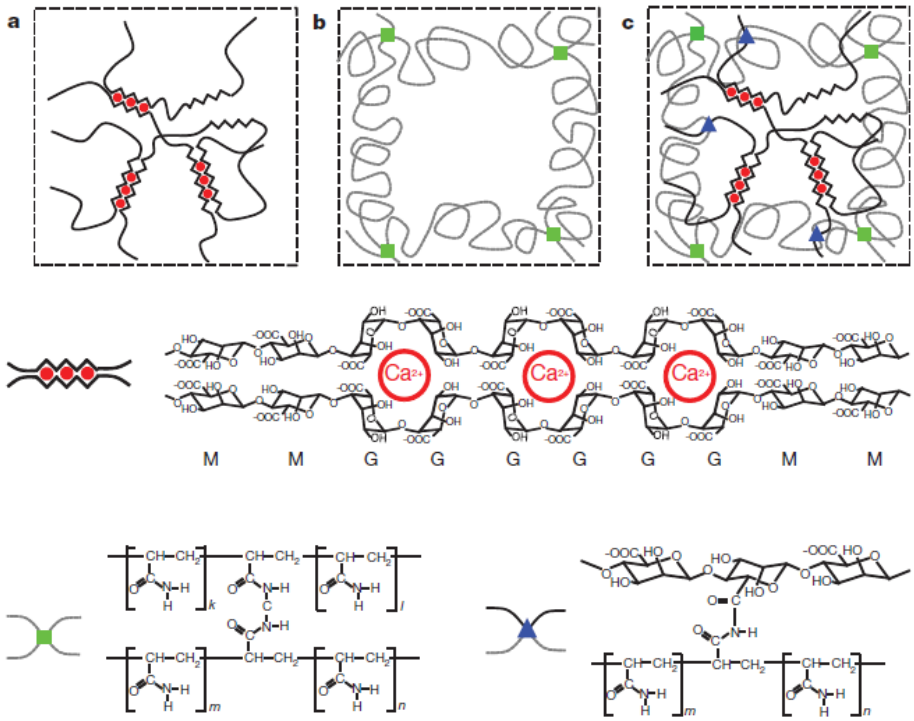

Figure 1.2 Double network hydrogels reported by J.-Y Sun and his team[4]

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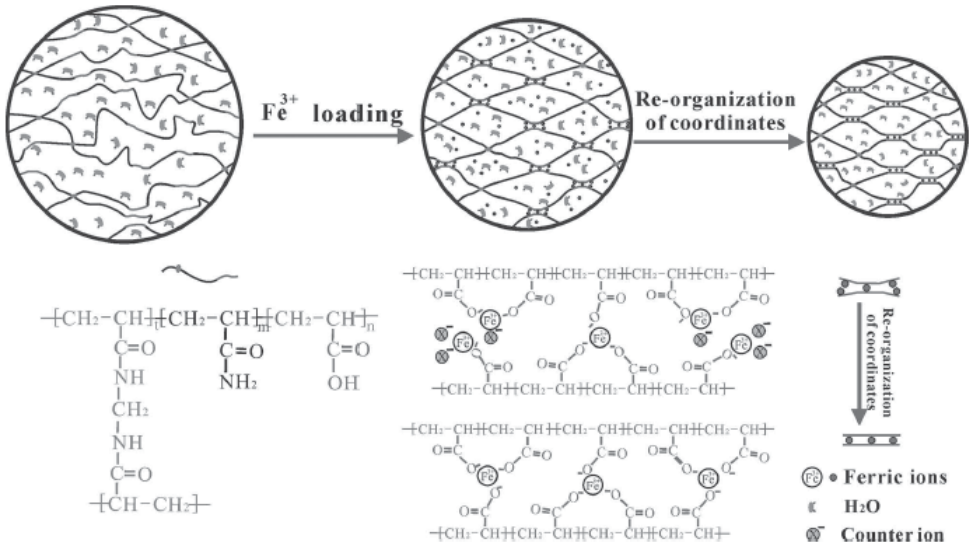

Figure 1.3 Dual crosslinking of PAAc hydrogel reported by P. Lin[2]

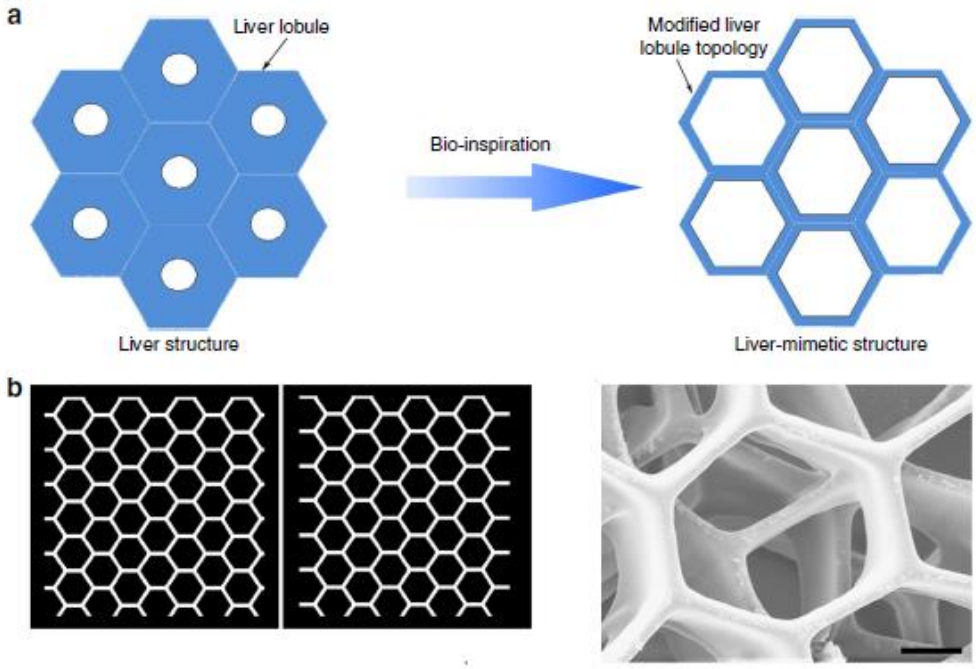

Figure 1.4 3D printed liver-like hydrogel structure proposed by M. Gou[3]

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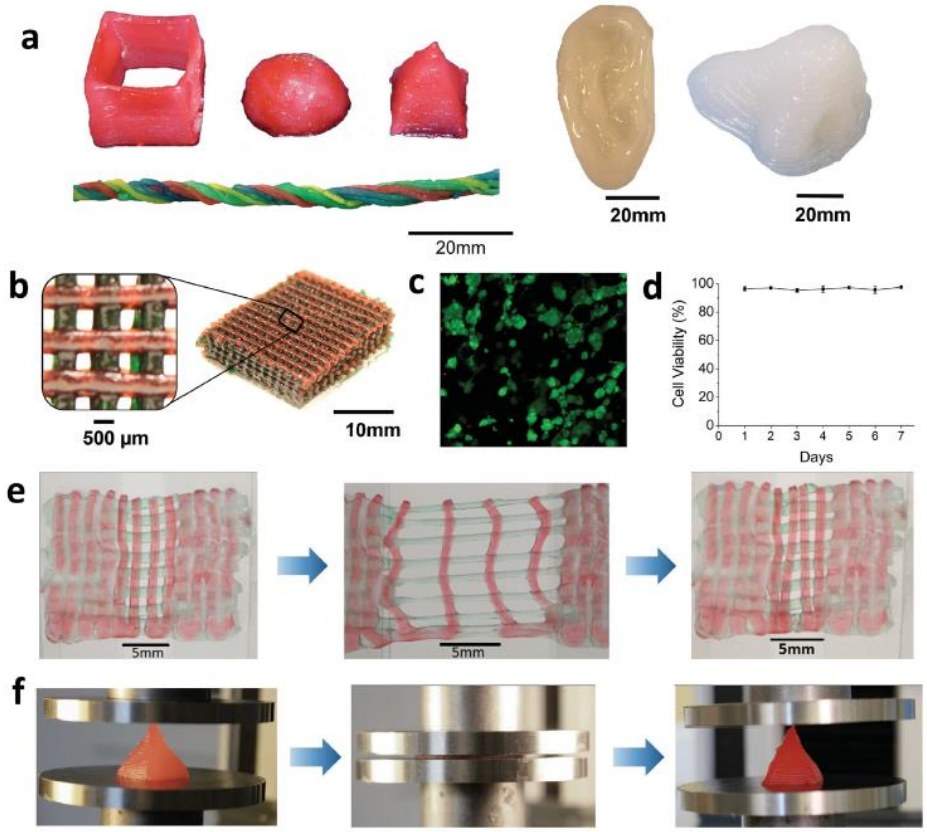


Figure 1.5 3D printed PEGDA/alginate hydrogel studied by S. Hong[5]

2. Theoretical backgrounds

2.1 Electroactive mechanism of polyelectrolyte

The polyelectrolyte hydrogels could be divided into two categories: anionic hydrogels such as PSPA and cationic hydrogels such as PAETMAC[18]. Anionic polyelectrolyte hydrogels consist of two ionic parts which are mobile cations and immobile anions. The anions are fixed in the polymer chains and maintain their positions within the solvent while the cations are freely movable inside the solvent. Thus, when an electric field is applied on the polyelectrolyte hydrogels, the mobile cations move toward the cathode whereas the anionic polymer chains are fixed on their original positions. In this condition, cathode-side portion of the hydrogel loses the cations and the anode-side portion cannot lose the anions. Moving of the ions causes osmotic pressure difference at the surfaces between the hydrogel and the electrolyte solution. As a result, cathode-side portion of the hydrogel deswells causing the bending motion of the hydrogel[13]. Cationic polyelectrolyte hydrogels bend toward anode by similar mechanism.

2.2 Role of macromolecules as a crosslinker

A crosslinker polymerizes with monomers forming cross-linked polymer network rather than linear chains of the polymer[19]. One of the widely used crosslinkers in biochemistry is *N,N*-methylenebisacrylamide (MBAA). MBAA crosslinks two linear chains at a single point. Density of the crosslinker influences mechanical properties of hydrogels, especially stretchability and Young's modulus are in a complementary relationship. High density of MBAA crosslinker densifies polymer networks causing hydrogels' stiffening. Thus, Young's modulus increases while stretchability decreases. The point of the 3D structural synthesis of the hydrogels is the balance of the two mechanical properties, *i.e.*, crosslinking density.

Unfortunately, stretchability of hydrogels have another problem for 3D structural synthesis, which needs fast polymerization in order to build supportable solid form. Polymerization ends in a brief time, limiting growth of polymer. Short chain length of polymer provides small amount of elastic energy which causes macroscopic elasticity drop and induces incompatibility of high elastic

modulus with high stretchability. These problems make electroactive hydrogels too brittle to be synthesized in 3D structures.

Therefore, a macromolecule as a crosslinker was proposed. Hyaluronic acid, one of the commonly used biomolecules, is polysaccharide which is naturally polymerized monosaccharide. D-glucuronic acid and D-N-acetylglucosamine is alternatively polymerized which have a lot of hydroxyl group (-OH) and carboxylic group (-COOH). At prior studies, many researchers succeeded in attaching vinyl group to the macromolecule by triggering ring opening reaction of the glycidyl methacrylate with catalysts, triethylamine and tetrabutylammonium bromide[20-22]. The studies showed that maximum 24 % of repeat unit of hyaluronic acid is methacrylated[23]. Thus, 1 out of 4 repeat has vinyl group, *i.e.* polymerizable group. When the macromolecule is modified with vinyl group, the macromolecule becomes crosslinkable state.

In this research, the modified hyaluronic acid was used as a crosslinker. Methacrylated hyaluronic acid (MA-HA) of mean molecular weight of 2,000,000 Da consists of nearly 5000 repeat unit and can complement the mechanical shortage of the electroactive hydrogels caused by short polymerization time and high MBAA concentration. Pre-

developed long chains of hyaluronic acid compensate short chains caused by short polymerization time and multi crosslinking points provide strong polymer networks.

2.3 3D printing method

3D printing has been developed for 35 years, many methods exist[5]. In 3D printing of polymer, 2 main ways exist: the one is squeezing polymers at high temperature in a 3D structure[16, 17] and the other is polymerizing monomer solution resin in a 3D structure using UV ray. Since hydrogels do not have thermoplasticity the latter one was chosen for 3D printing of smart hydrogels. In this research, DLP (Digital Light Processing) method was chosen[3, 24].

DLP method consists of 3 parts which are a UV source, a DMD chip and a stage in monomer solution bath. The UV source emits ultra-violet light with a proper reaction wavelength of photoinitiator. The DMD chip (Digital Micromirror Device chip) projects the shape of cross-section of the object layer by layer. The stage in the bath containing monomer solution is ascending layer by layer. On the stage, the hydrogel structure growths as an integration of 2D planes making a 3D structure.

DLP method has some advantages for hydrogel's printing, particularly smart hydrogels. First of all, printing speed is fast enough for water not to be vaporized within the synthesize period. Whereas FDM (Fused Deposition

Modeling) and SLA (Stereolithogrphy) methods use accumulation of 1 dimensional line and laser delaying overall synthesis time, DLP uses accumulation of 2 dimensional plane projector which completes the 3D structure faster. Secondly, DLP method does not ruin the functionalities of smart hydrogels. Since hydrogels do not have thermoplasticity, additives which make the monomer solution highly viscous are necessary for squeezing methods. Such additives disrupt the electroactive mechanism and other smartness functions. Finally, DLP method is relatively inexpensive to build up an apparatus than SLA method which uses UV laser.

2.4 Water soluble 365nm Photoinitiator

Photoinitiation is compulsory for 3D printing technology, polymerized by UV lay in a brief time[25]. DLP 3D printing of hydrogels needs a photoinitiator for radical polymerization, a chemical which let monomer solutions be hydrogels under ultraviolet environment. Under the light with specific wavelength, the photoinitiator molecules are divided into two radical molecules, and those radicals initiate polymerization of the monomers followed by chain reaction. Unfortunately, current DMD chips only operate in wavelengths of 365 nm or higher and no commercial photoinitiator is water soluble and reacts to 365 nm UV light. In the field of hydrogels' photo polymerization, Irgacure 2959 (2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone) is widely used, however the peak wavelength of UV absorption of Irgacure 2959 is 324 nm, not appropriate for DLP method.

Thus, LAP (Lithium phenyl-2,4,6-trimethylbenzoyl phosphinate) photoinitiator was introduced. LAP was first synthesized at 1991 by Majima Tetsuro, with 2 steps reaction. As the first step, methyl phenyl-2,4,6-trimethylbenzoylphosphinate (MAP) was prepared for 370 nm UV light reactive photo-initiator, by Michaelis-

Arbuzov reation. After the first reaction, MAP was ionized by lithium bromide in order to make MAP water-soluble. This ionized MAP, LAP is ready to be used as a 365 nm UV reactive photoinitiator. In this research, LAP was synthesized via the recipe of Majima's method and used properly.

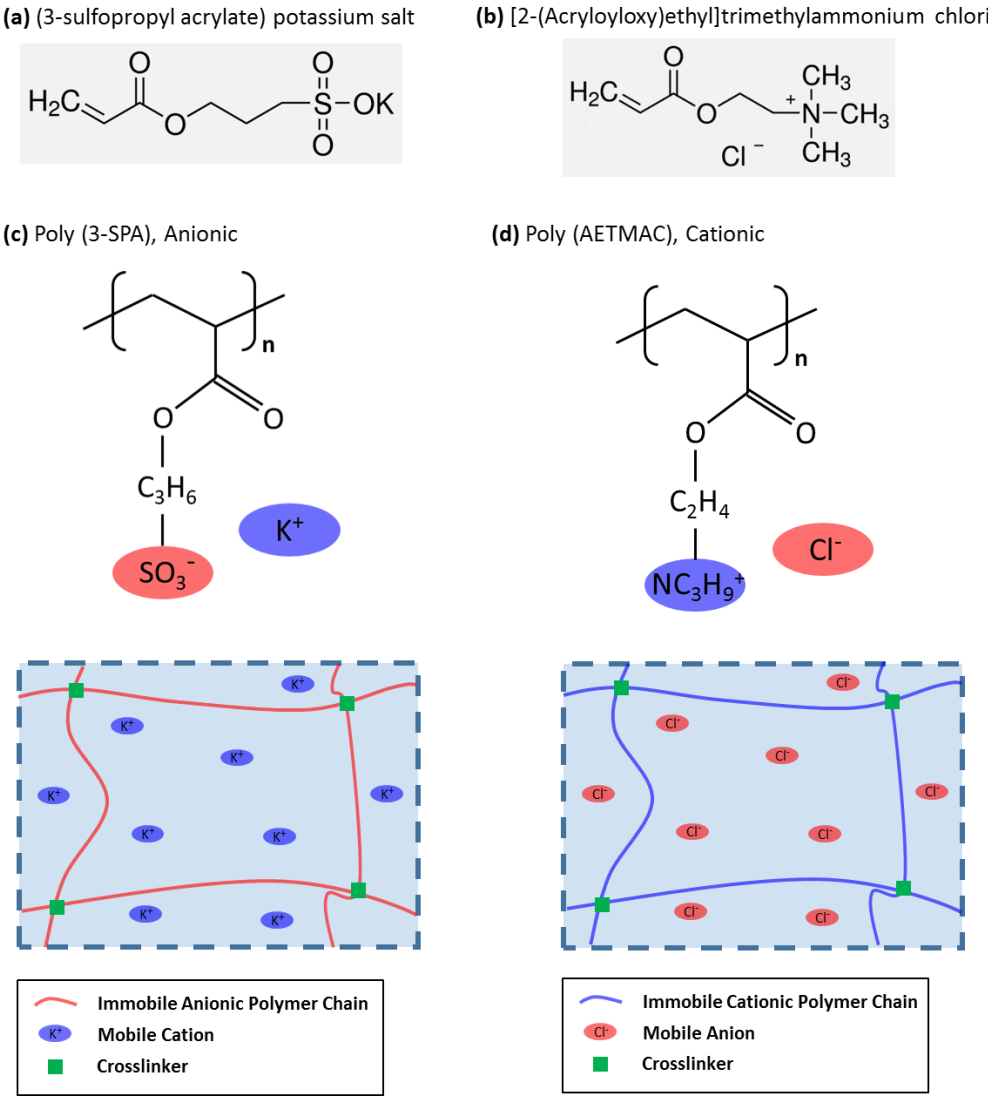


Figure 2.1 Polyelectrolyte hydrogels. (a) SPA molecule (b) AETMAC molecule (c) schematics of PSPA hydrogel (d) schematic of PAETMAC hydrogel

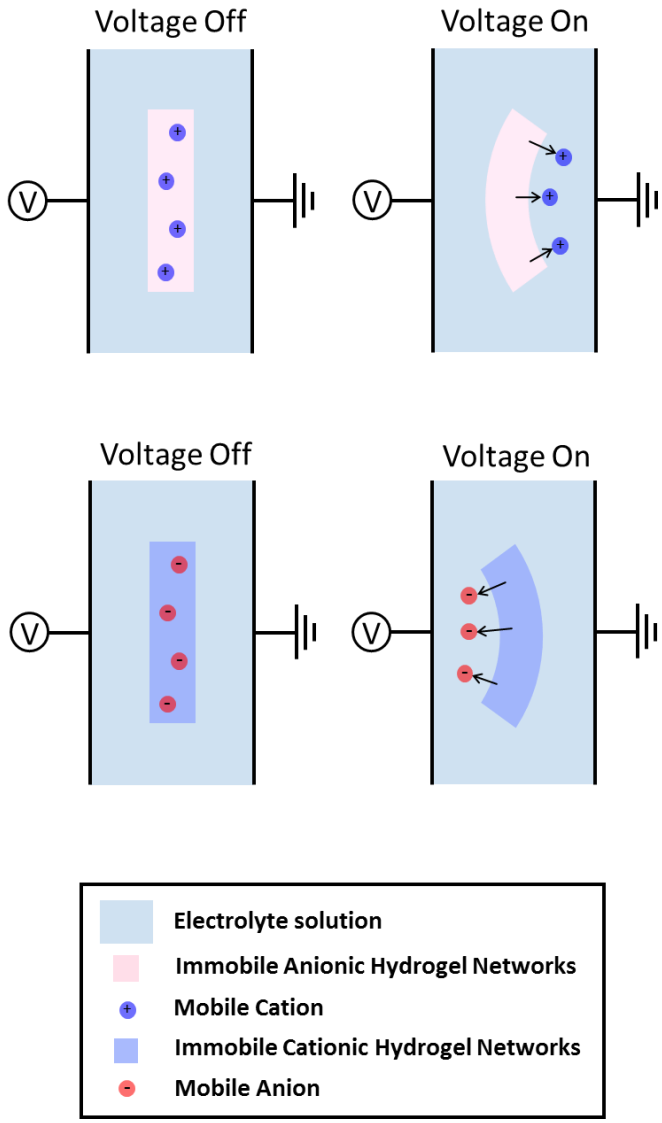


Figure 2.2 Electroactive mechanism of polyelectrolyte hydrogels

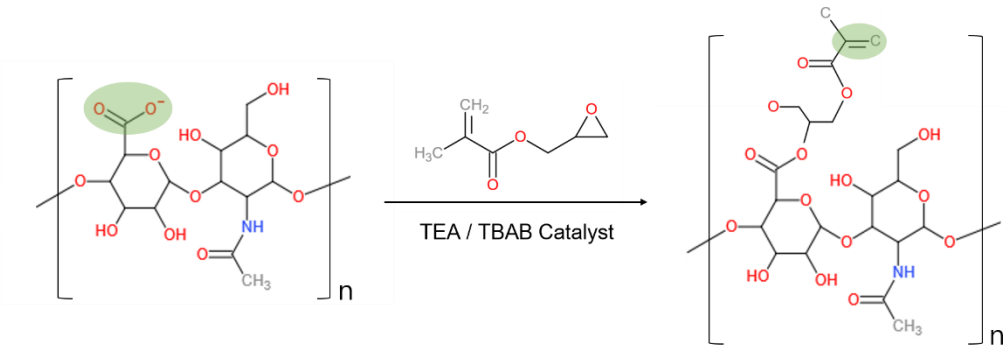


Figure 2.3 Methacrylation of hyaluronic acid using glycidyl methacrylate

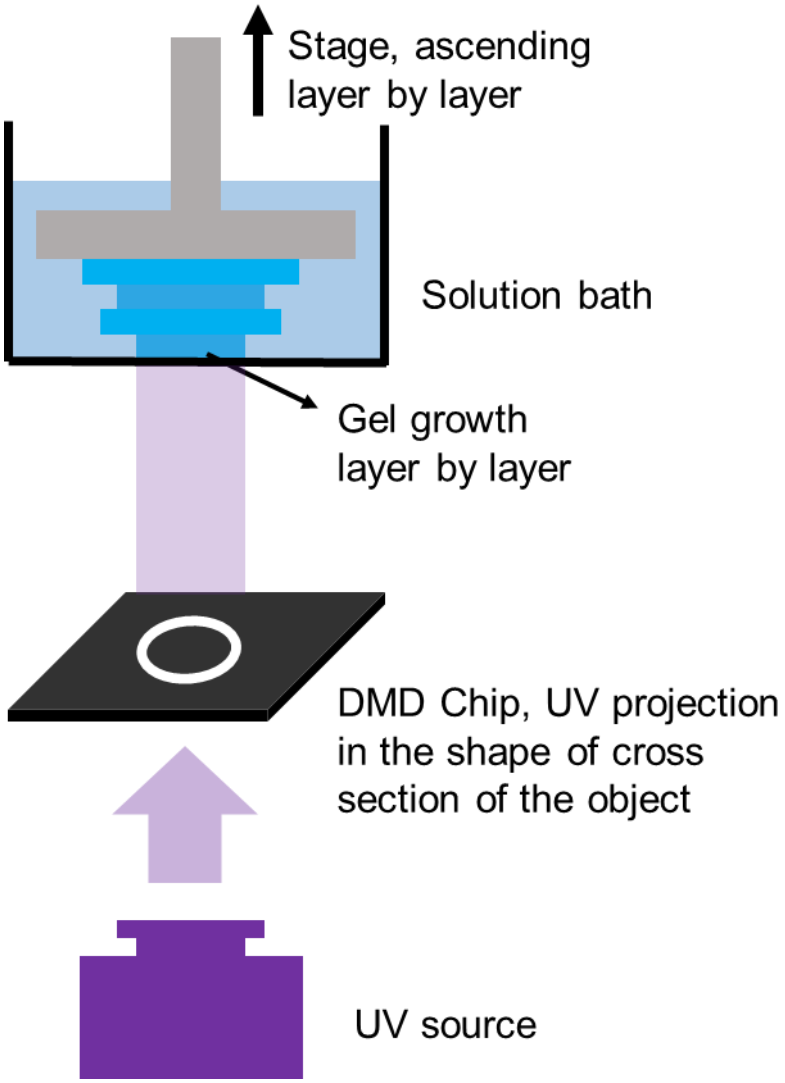


Figure 2.4 Schematics of DLP 3D printer

3. Experimental

3.1 Photoinitiator synthesis

Non-commercial photo-initiator LAP was synthesized in order to 3D printing of the hydrogels. The recipe referred to the prior study[26]. Dimethyl phenylphosphonite (Alfa Aesar, 99%, used without further purification) 1.905 ml and 2,4,6-trimethylbenzoyl chloride (Alfa Aesar, 99%, use without further purification) 2 ml was mixed in a molar ratio of 1:1 and the mixture was stirred for 24h at room temperature. After stirring, the mixture was put into 70 ml of 2-butanone (Sigma Aldrich, 99.9%) containing 4.24 g of LiBr (Sigma Aldrich, 99.9%), reacted at 50 ° C for 4 hours. During the reaction, white plaster-like chemical precipitated. White precipitation was separated from the mixture by vacuum filter and washed by an excess of 2-butanone 2~3 times. The filtered powder was dried out in vacuum for a day and ready to use.

3.2 Hyaluronic acid methacrylation

For the preparation of a macromolecule crosslinker, methacrylated hyaluronic acid was synthesized using the recipe of prior studies[27]. 0.5 g of sodium hyaluronate with average molecular weight of 2,000,000 (Kikkoman) was dissolved in 100 ml of 1M PBS solution (Sigma Aldrich, pH 7.4). 1.95g of triethylamine (Sigma Aldrich, 99.9%) and 1.95g of tetrabutylammonium bromide (Sigma Aldrich, 99.9%) were added to the solution in order to accelerate of ring opening reaction. After stirring the solution for 20 minute with 300 RPM, 2.5 ml of glycidyl methacrylate (Sigma Aldrich, 99.9%) as methacrylation reagent was added to the solution. The solution reacted for 24 hours at room temperature followed by 2 hours reaction at 60 ° C. The methacrylated hyaluronic acid was precipitated and washed in an excess of acetone 3 times. After evaporation of acetone, the powder was dissolved in water and freeze dried for 1 week and ready to use.

3.3 Electroactive hydrogels

To measure mechanical properties, PSPA hydrogel as an anionic hydrogel and PAETMAC hydrogel as a cationic hydrogel were synthesized. 3-sulfopropylacrylate potassium salt (Sigma-Aldrich, 99%) and 2-(acryloyloxy)ethyl trimethylammonium chloride (Sigma-Aldrich, 80 wt% in H_2O) were used as monomers. Monomer concentration of PSPA hydrogel was 1, 2, 3 M, and PAETMAC was used as it stood. Concentrations of MBAA (Sigma Aldrich, 99.9%) and methacrylated hyaluronic acid as crosslinkers were various to find out the mechanical properties change. LAP as a photoinitiator was added to all solutions in a concentration of 0.8 wt% of monomer. Irgacure 2959 (Sigma Aldrich, 98%) and ammonium persulfate (Sigma Aldrich, ≥98%) were used as polymerization initiators, photo and thermal initiator respectively. In cases of need for clarity, methylene blue (Sigma Aldrich, 99.9%) was added with a concentration of 0.05 wt% of monomer as a dye. The monomer solutions were poured in an acrylic plate with a size of 20 × 10 × 5 mm^3 and polymerized in 365 nm UV chamber (UVP, CL-1000) for 30 secs. After polymerization, the hydrogels were soaked in 0.1 M NaCl aqueous solution till they reached swelling equilibrium under corresponding osmotic

pressure. For the printing solutions, ratio of LAP photoinitiator and MBAA crosslinker varied in order to balance polymerization time, mechanical properties and bending degree of 3D structured hydrogels.

Prepared PSPA, PAETMAC electroactive hydrogels were laid in acrylic bath containing Normal Tyrode's solution with platinum coated titanium electrodes (Baoji Qixin Titanium Co., Ltd.) at both sides of the bath. In order to check the electroactivity, an electric field of 2.5 V/cm was applied to the bath containing the hydrogels for 30 secs. The angle between the extension lines of the top and the bottom segments of the bended rectangular was defined as bending degree.

To prepare Normal Tyrode's solution, 8.0 g of sodium chloride (DAEJUNG, 99.9%), 0.2 g of potassium chloride (DAEJUNG, 99.8%), 0.2 g of calcium chloride (DAEJUNG, 99.9%), 0.1 g of magnesium chloride (DAEJUNG, 99.8%), 0.05 g of sodium dihydrogen phosphate (Sigma Aldrich, 99%), 1.0 g of sodium bicarbonate (Sigma Aldrich, 99.9%) and 1.0 g of dextrose (Sigma Aldrich, 99%) were dissolved in 1 L of deionized water. pH of the solution was set to 6.5 with hydrochloric acid and sodium hydroxide 1 M solution.

3.4 Measuring mechanical properties

Mechanical tests of the polyelectrolyte hydrogels, *i.e.* PSPA, PAETMAC hydrogels, were carried out with a mechanical test apparatus (instron®, 3342 machine) using 50 N load cell. For nominal compression tests, the gel was synthesized in a 40 × 40 × 10 mm^3 glass mold, and cut with a laser cutter (Universal Laser Systerm, VLS3.50). Compression tests were conducted with disc shaped hydrogels which have 10 mm diameter and 10 mm thickness. Each specimen of the gel was compressed until the sample was broken. The speed of compression of the load cell tip was 0.1 mm/sec. For tensile tests, electroactive hydrogels with a size of 15 × 40 × 3 mm^3 was prepared. To prevent sliding between clips and gel samples, acrylic plates were attached to the top and the bottom of the samples with a superglue to provide stiff grip area for a tensile test. Gel samples were loaded uniaxially after gluing. The tensile stretch speed of the gel was 0.1 mm/sec.

3.5 3D printing of hydrogels

DLP 3D printing apparatus was constructed by modifying a commercial DLP printer (Illuminaid, Litho 3D printer). 100 W metal-halide lamp was used as a light source, and a DMD chip provided from the company was used without further optimization. The x, y, z axis resolution of 3D printing was 50 micrometers and maximum build-up size was 56 × 72 × 100 mm^3. Hydrophobic coated slide glass (Matsunami, 76 × 52 mm^2) was attached on the stage where the synthesized structure grows in order to easily lift up the in-process structure and easily remove the final production. The bath containing the monomer solution was covered by PDMS (Sylgard, 184) in order to detach the structure in the process. The basic environment of 3D mechanical movement is driven under a program with G code made for the apparatus only.

DLP method for 3D printing of hydrogels has mainly 3 variables for optimization. UV light exposure time at each layer, concentration of the photoinitiator and ratios of crosslinkers are those. To confirm that only first 50 micrometers depth of the solution from the UV light source is polymerized, *i.e.* to control the penetration depth, UV light exposure time was optimized in a time scale of microseconds. Ratio of LAP photoinitiator and MBAA

crosslinker varied to find proper characteristics because it influences polymerization time, penetration depth, mechanical properties and electroactivity.

In order to optimize the variables in the 3D printing apparatus, poly acrylamide hydrogels were synthesized. Initial values of the optimization were described: 0.15 mol of acrylamide (Sigma Aldrich, 99.7%) and 7.5 mmol of MBAA and 0.1 g of methacrylated hyaluronic acid was poured into water 100 ml of total volume. The photoinitiator LAP was added with a concentration of 0.5 mol% of total monomer.

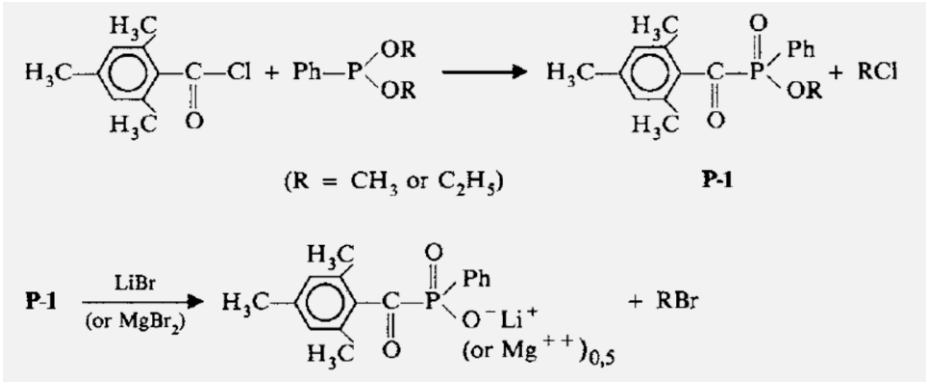


Figure 3.1 Synthesis of LAP photoinitiator

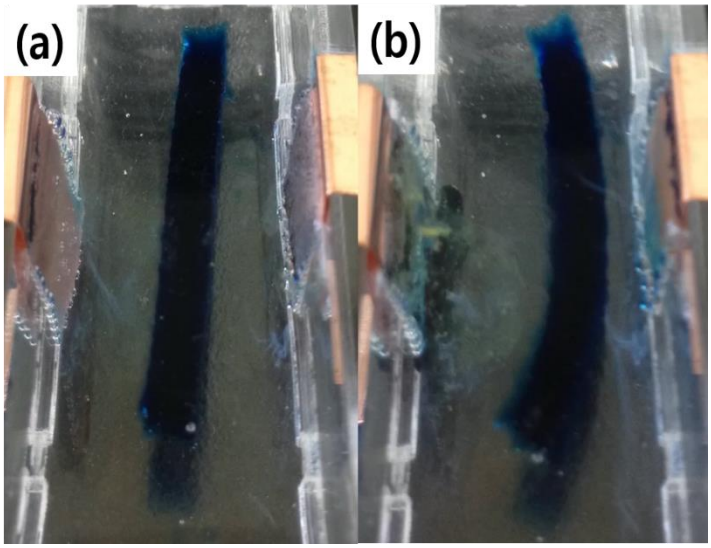


Figure 3.2 Bending of PSPA hydrogel under an electric field

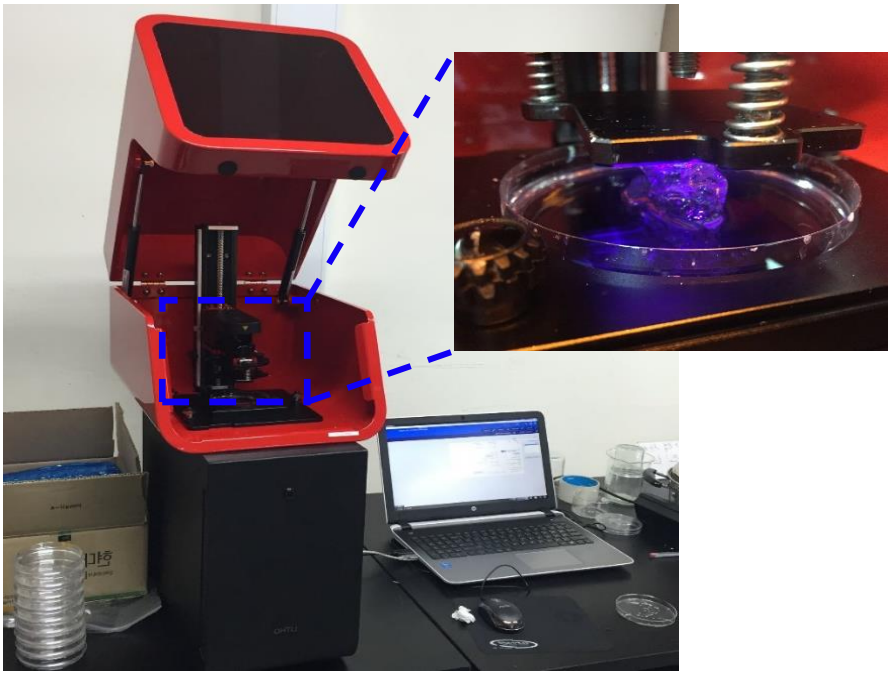


Figure 3.3 DLP 3D printing apparatus

4. Results and discussion

4.1 LAP Photoinitiator

The photoinitiator was successfully synthesized in two-step reaction. Synthesized LAP photoinitiator and commercial photoinitiator Irgacure 2959 were dissolved in water with a concentration of 1 μmol respectively for UV-Visible spectroscopy (Agilent, Cary-60). The photoinitiator molecules absorb particular wavelength of light and are divided into 2 radical pieces, causing radical polymerization. The UV-Visible spectroscopy data show the active wavelength of the photoinitiators. Commercial Irgacure 2959 has the peak value of absorbance at 280 nm UV-B wavelength and absorbance at 365 nm which is used as a light source in DLP method is only 0.04 %. On the other hand, synthesized LAP photoinitiator has the peak value of absorbance at 374 nm wavelength, which was proved as a proper photoinitiator of DLP 3D printing of hydrogels.

To check the hardenability of monomer solutions by polymerization induced by LAP photoinitiator, real-time rheometer experiments measuring storage modulus were conducted. 20 wt% acrylamide solution with 0.05 wt% MBAA crosslinker was used. LAP 0.1, 0.2, 0.4, 0.8, 1.6 wt%

of total monomer were added respectively to the solutions as a photoinitiator. Fixed quantities of the solutions were laid down on the stage of rheometer, and UV lamp (UVP, 365 nm, 8 W) radiated UV light to the solutions while rheometer was working. Storage modulus of the samples stands for the elasticity of non-Newtonian viscoelastic fluid.

For the first 40 secs, all of the samples maintained their storage moduli as about 10 Pa, which means that the samples were in solution states, not solidified. Storage modulus curve of the sample with the highest concentration of LAP started to rise at 45 sec, went over 100 Pa within 10 sec from the rising point. The values of the rising points, *i.e.* gelation points, and reaching to 100 Pa points delayed along with the decrease of the concentration of LAP photoinitiator. For the DLP 3D printing, hardening of the solution has to finish within a short time interval. The time interval between the rising point and the 100 Pa point needs to be short. The interval was shortened with high concentration of the photoinitiator.

Unfortunately, storage modulus at 300 sec point decreased along with increase of the concentration of the photoinitiator. The highest concentration sample of 1.6 wt%

had the lowest storage modulus and the lowest concentration sample of 0.2 wt% had the highest storage modulus. Since storage modulus stands for the elastic portion of viscoelastic materials, lower storage modulus indicates the lack of elasticity. Lack of elasticity enforces the hydrogel to be plastically deformable, making the hydrogels brittle. Brittleness in hydrogels hinders 3D printing structure. Thus, unconditionally high concentration of the photoinitiator does not satisfy the printing conditions. 0.8 wt% sample was chosen for the printing process.

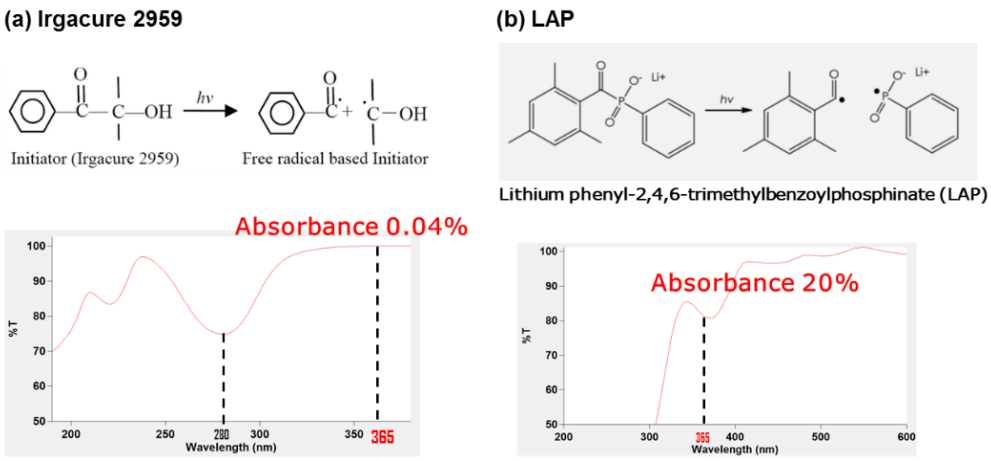


Figure 4.1 Comparison of (a) commercial photoinitiator Irgacure 2959 and (b) synthesized photoinitiator LAP

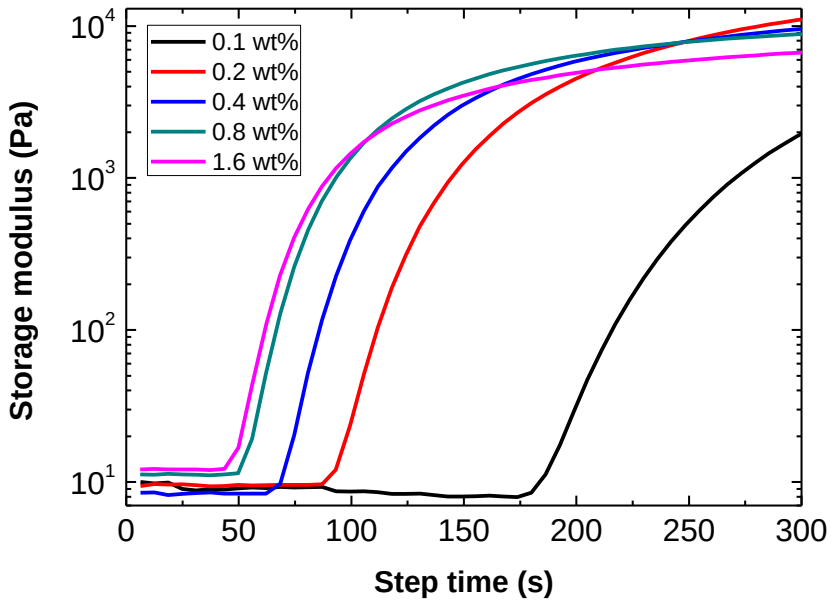


Figure 4.2 Gelation time of PAAm hydrogels initiated by LAP

4.2 Effect of methacrylated hyaluronic acid

4.2.1 Effect of methacrylated hyaluronic acid

To check the effect of methacrylated hyaluronic acid as a crosslinker, PSPA anionic hydrogels were synthesized with two crosslinker, MBAA and methacrylated hyaluronic acid. Using photoinitiator LAP with a concentration of 0.8 wt% of the monomer in all experiments, 1M and 2M SPA hydrogels were synthesized. Each sample was crosslinked by 0.1 wt% of MBAA or 0.1 wt% of methacrylate hyaluronic acid.

The effect of crosslinker was remarkable. Crosslinked by MBAA crosslinker shows high elastic modulus and low stretchability. 1 M and 2 M samples with MBAA crosslinker had 10 kPa, 31 kPa elastic modulus and 261 %, 145 % stretchability respectively. Contrastively, each sample with MA-HA crosslinker had 4.0 kPa, 0.7 kPa elastic modulus and 850 %, 700 % stretchability. The methacrylated hyaluronic acid can play a role of crosslinker, providing enormous stretchability to brittle hydrogels while the elastic modulus decreases.

MBAA and MA-HA were used at the same sample for mutual complement of their advantage. Anionic PSPA

hydrogel and cationic PAETMAC hydrogel were synthesized in 2 M solution. The same concentration of photoinitiator LAP with the prior experiments was added to the solutions. PSPA and PAETMAC both hydrogels showed compensated properties in the samples of MBAA + MA-HA crosslinkers. PSPA 2M hydrogel with MBAA 1 wt% and MA-HA 1 wt% showed 18 kPa elastic modulus and 270 % stretchability. These compensated properties did not appear in the single crosslinker samples. This means that well-mixture of two complementary crosslinkers can accomplish the target we need to do 3D printing of the electroactive hydrogels.

4.2.2 Demand of mechanical properties for 3D printing

There are minimum values of elastic modulus and stretchability. Prepared DLP apparatus adopts bottom-up method that 3D structure grows upside down under the stage. 3D structure synthesized below the stage is inevitably dragged down by gravity. The problem is that the 3D coordinates could go awry if elastic modulus of the material is small. Pre-solidified parts deviate from the right coordination because of gravity.

According to the definition of elastic modulus ($E = {}^{\sigma}/_{\varepsilon}$), the minimum elastic modulus could be calculated by assuming that 1 cm^3 cubic-shaped hydrogel grows via 3D apparatus. To confirm the degree of precession of the printing, strain value (ε) of the very end of the cubic has to be smaller than 10 μm, which is 20% of the mechanical resolution. Mass of 1cm^3 cubic-shaped hydrogel is about 1 g since it mostly consists of water and acceleration of gravity is 9.8 m/s^2. σ and ε value can be calculated from the numbers.

$$\sigma = \frac{\mathrm{N}}{\mathrm{A}} = \frac{9.8\ g{\cdot}m/s^2}{1\ cm^2} = 98\ N/m^2 \quad and\ \varepsilon = \frac{\Delta\ l}{l} \leq \frac{10\ \mu m}{1\ cm} = 0.001$$

According to the calculation, when the height of the structure is under 1 cm, 98 kPa of elastic modulus of the

material guarantees the accuracy of 3D printing.

Thus, pure PSPA and PAETMAC hydrogels needed to be mechanically enhanced. The problem is that electroactive hydrogels including PSPA and PAETMAC has excessively low mechanical properties. As a solution of the problem, acrylamide was added as a co-monomer with the monomers of PSPA, PAETMAC hydrogels.

Total monomer concentration was 2 M in all samples, 20 mol% of total monomer was substituted by acrylamide. Since the elecroactivity of polyelectrolyte did not ruined by addition of non-charged monomers when the difference of ionic strength between the inside of hydrogel networks and the outside of hydrogel networks, additional monomer was allowed. Concentration of the photoinitiator LAP was identical, 0.8 wt% of monomer. MBAA crosslinker 0.1 wt% and MA-HA crosslinker 0, 0.1 wt%, 0.2 wt% was added to the samples respectively.

The experiments provided an encouraging result. While previous samples with pure PSPA and PAETMAC hydrogels had insufficient mechanical properties to be 3D structurally synthesized, the copolymer hydrogels showed sufficient values of elastic modulus and stretchability. Especially, the sample with 0.1 wt% of MBAA and 0.1 wt% of MA-HA crosslinkers showed 102, 98 kPa of elastic

modulus in PSPA and PAETMAC hydrogels respectively, which value is sufficient to guarantee the accuracy of 3D printing apparatus. Also, stretchability was enhanced remarkably with MA-HA crosslinker. Only 0.1 wt% of an additive make the electroactive hydrogels suitable for 3D printing.

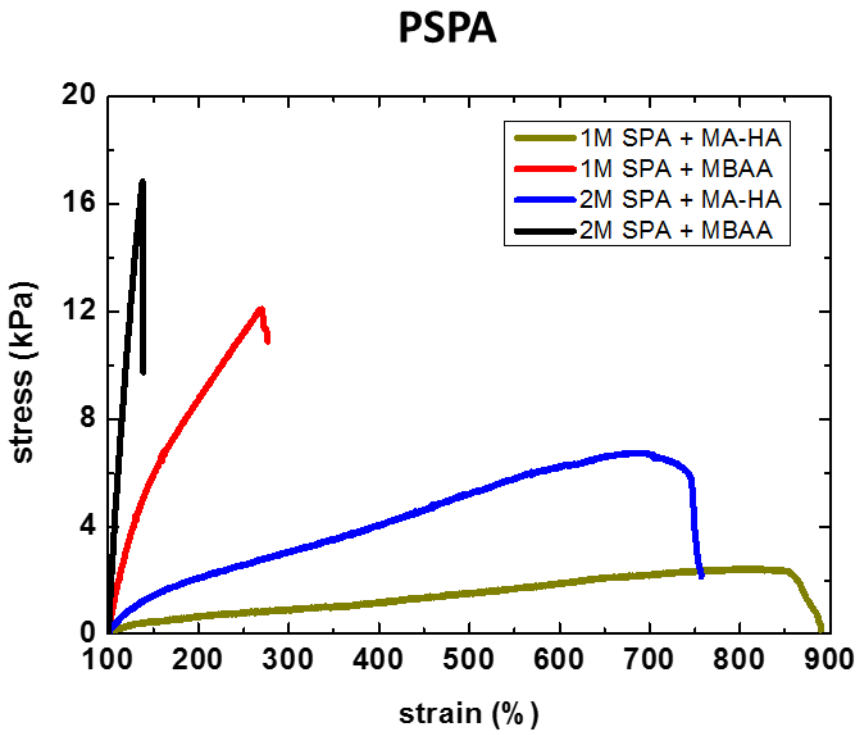


Figure 4.3 Stretch tests of PSPA hydrogels

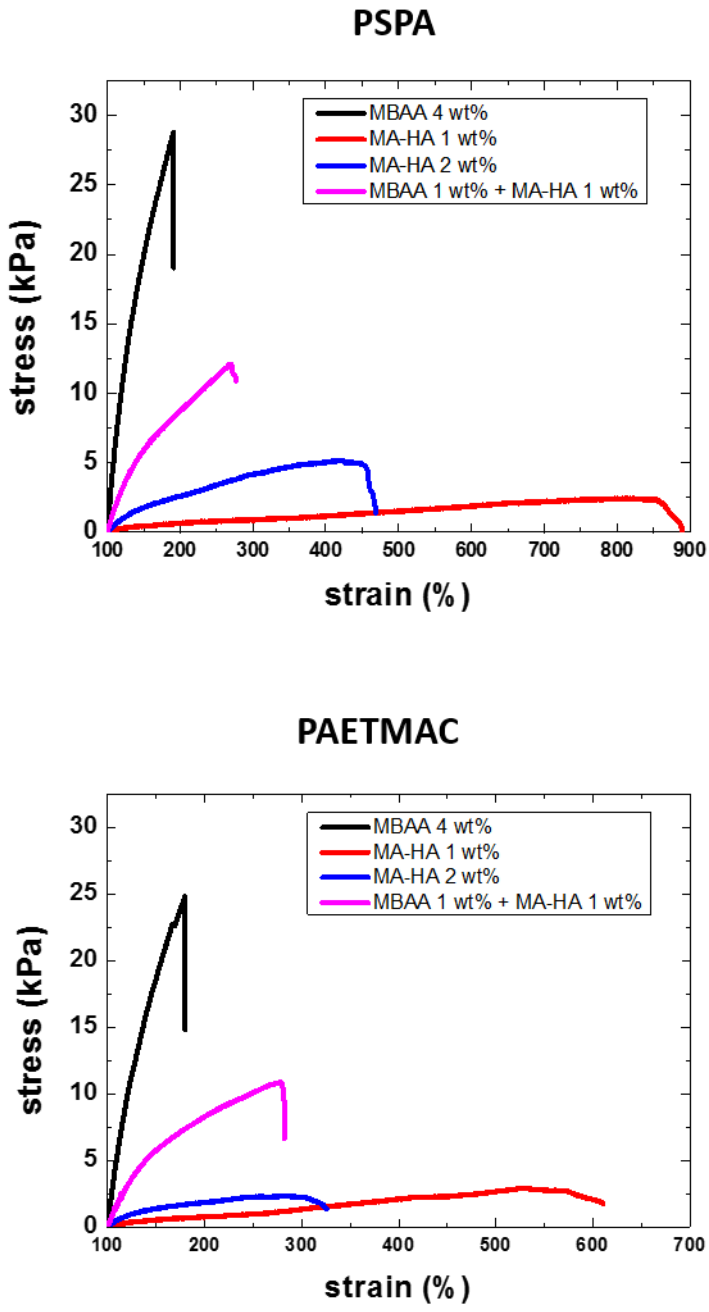


Figure 4.4 Stretch tests of PSPA and PAETMAC hydrogels with dual crosslinkers

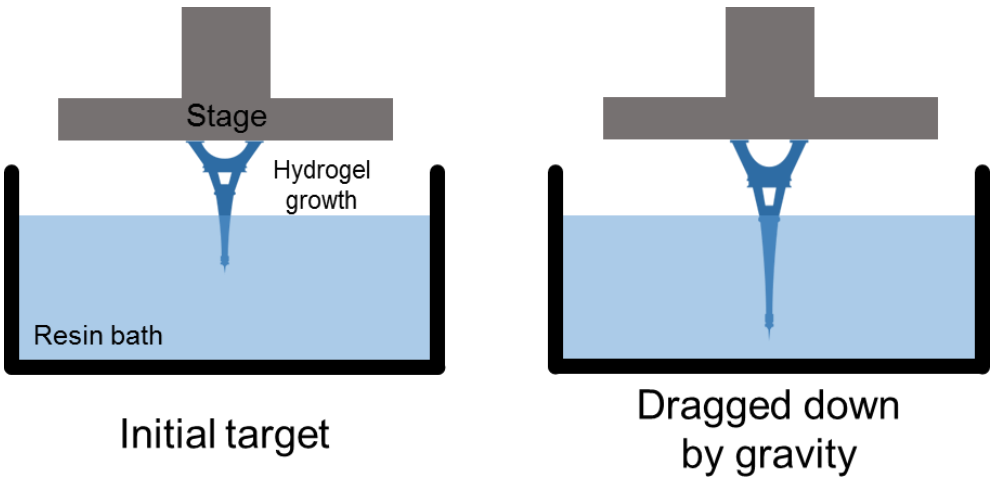


Figure 4.5 Dragging of hydrogels caused by gravity

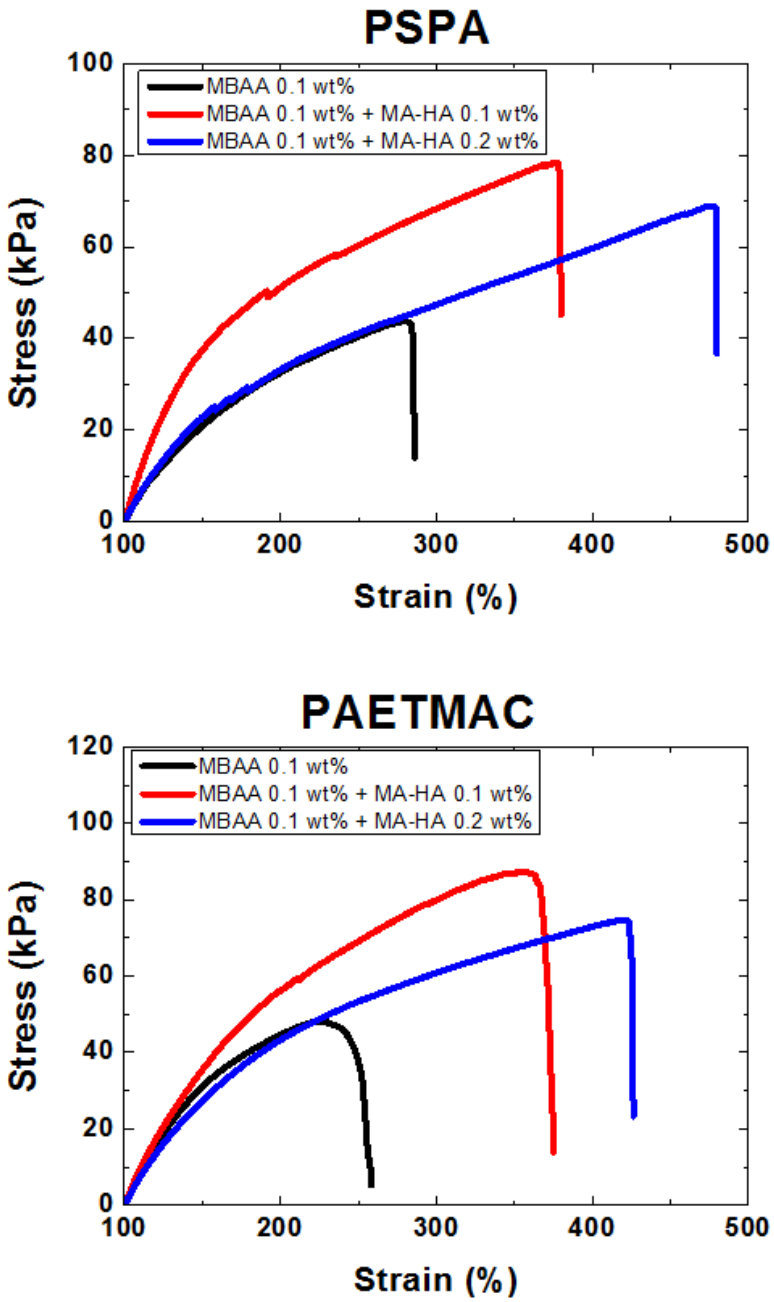


Figure 4.6 Stretch tests of P(SPA-AAm) and P(AETMAC-AAM) hydrogels

4.3 Electroactivity

Electroactivity tests were conducted in Normal Tyrode's solution, which is similar to the human body fluid in terms of osmotic pressure and ionic balance. The overall salt concentration of the solution was about 150 mM. SPA with AAm hydrogels are synthesized in various crosslinker concentration. 2 M of total monomer concentration with a ratio of 2:8 for AAm:SPA were used and photoinitiator LAP with 0,8 wt% of total monomer was added.

The result showed that the electroactivity existed in all samples regardless of sort and concentration of crosslinker. The bending angles varied from 52 degrees in the first sample to 25 degrees in the eighth sample. Only the sample with 0.1 wt% of methacrylated hyaluronic acid as a crosslinker showed nothing, but it is because the softness of the hydrogel which cannot be handled.

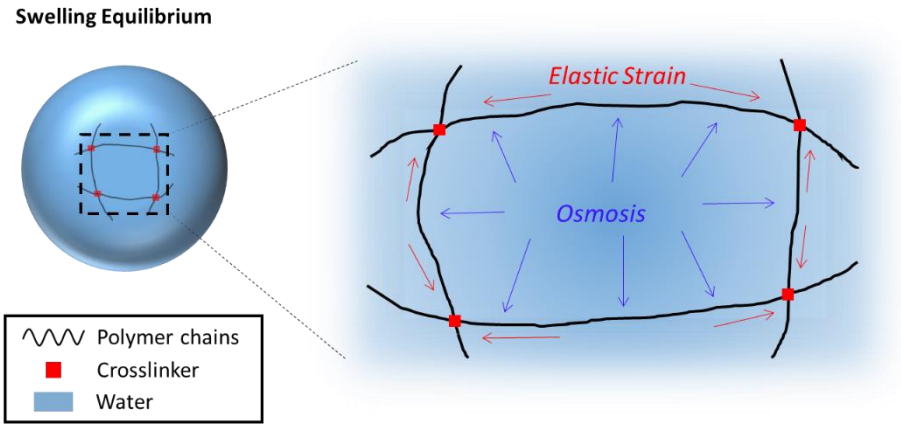
A tendency was found in the experiment. The sample with high elastic modulus showed relatively low bending angle regardless of type of crosslinker and stretchability. Those tendency arises from the bending mechanism. The bending occurs when the swelling abilities in both ends differ from each other, for instance one side swells whereas the other side deswells. The swelling ability comes from the

equilibrium between the elasticity of polymer chains and osmotic pressure.

$$W_{eq} = W_{elastic} + W_{osmotic}$$

Where $W_{elastic}$ is elastic energy which induces contraction of the hydrogel and $W_{osmotic}$ is osmotic energy which mainly induces swelling of the hydrogel. When an electric field is applied, both sides of the hydrogel have disparity in terms of osmotic pressure while the hydrogel's elasticity maintains. Bending angle of electroactive hydrogels gets larger when the ratio of W_{eq} of both sides of the hydrogel is greater.

When the elastic energy of the hydrogel is high, *i.e.* Young's modulus of the hydrogel is high, $W_{elastic}$ gets high value. High value of the hydrogel's $W_{elastic}$ minifies the ratio of W_{eq} of both sides of the hydrogel induced by difference of $W_{osmotic}$ under an electric field. The sample with high elastic modulus shows small difference between swelling ratios of both sides causing low bending angle under an electric field.



$$W_{eq.} = W_{elastic} + W_{osmotic}$$

Figure 4.7 Swelling of hydrogels

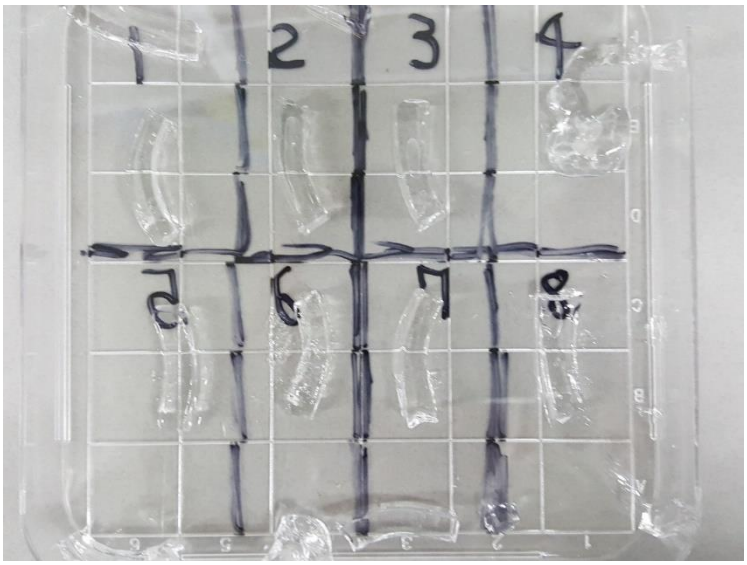


Figure 4.8 Electroactivity tests of mechanically enhanced P(SPA-AAm) hydrogel

4.4 3D printing of electroactive hydrogels

To train the DLP 3D printing method with hydrogels, acrylamide hydrogels were chosen, in the composition described in the experimental section.

The main technical problem of DLP printing method is adjusting penetration depth of UV light. Otherwise the other photo-sensitive polymer resins, the hydrogel resins are fully transparent, which means that intense UV light easily go through the solution and reach to surface of the solution. For 50 μm of resolution, only 50 μm of resin UV light meets first has to be gelated, however penetrated UV light gelates the whole vertical part of resin in a few microseconds after the first portion of the solution is gelated. Thus, UV light irradiation time has to be delicately modulated. In case of need, ecamsule, a UV absorber, was added to the solution in order to reduce penetrability of UV light.

To optimize the conditions of hydrogel printing, some products were manufactured. 3D modeling was designed with a software CATIA (Dassault Systems, V5R19). The emblem of Seoul National University was printed to check the x, y axis resolution of the hydrogels. Size of the sample was 40 × 40 mm^2. As shown in figure, the resolution of

hydrogels was about 100 μm. The difference between the limit of the machine, *i.e.* 50 μm and real value come from the distraction of UV light inside the resin solution.

The second figure was a vase. Diameter of the middle of the vase was 20 mm and height of the vase was 30 mm. Because of the UV penetration come up in the prior paragraph, the surface of the vase was fairly rough. Inside of the base was well constructed, thus water can be contained in the vase.

For the last sample, lens array was constructed to check the z axis resolution. Diameter of each lens was 5 mm. The lens array in Figure was fully swelled state in DI water, and lens diameter was about 10 mm. Despite the guaranteed mechanical limitation of z axis resolution was 50 μm, 10 μm of z axis movement was successfully operated. Lens array provided a user experience similar to commercial magnifying glass.

With the mechanically enhanced electroactive poly(AAm-SPA) hydrogel, electroactive Pisa tower was synthesized. For the clarity of observation, 0.05 wt% of methylene blue was added to the printing solution. Diameter of the main body was 8 mm, the bottom board was 15 mm. The structure was synthesized in tilted state as it is in Rome, 5.5 degrees from earth. The structure was laid

in a bath with 2 cm of width containing Normal Tyrode's solution as electrolyte. Pt-coated titanium electrodes were set to the both ends of the bath and 5V DC potential was applied for 30 secs to the electrodes. After 30 seconds, electroactive Pisa tower was straightened.

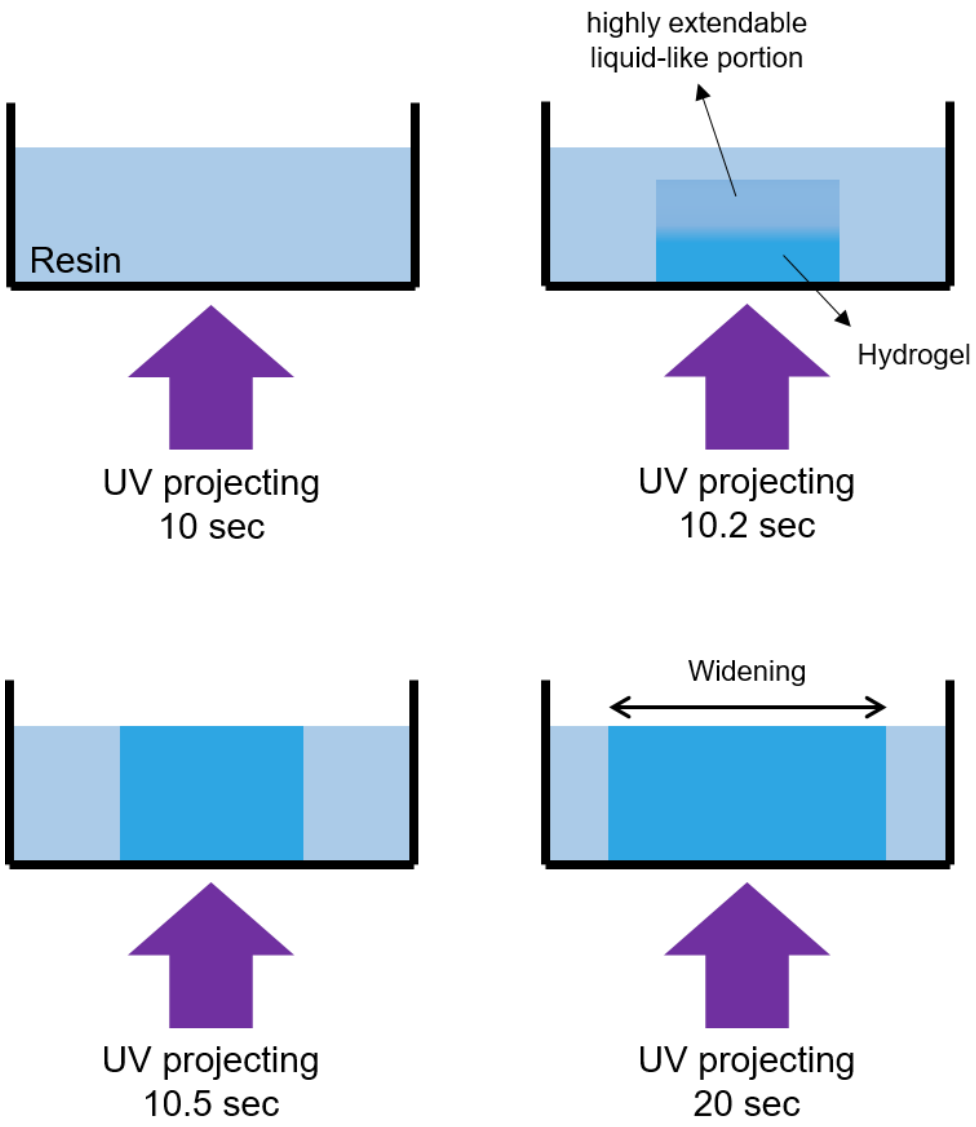


Figure 4.9 Penetration of UV to the resin, monomer solution

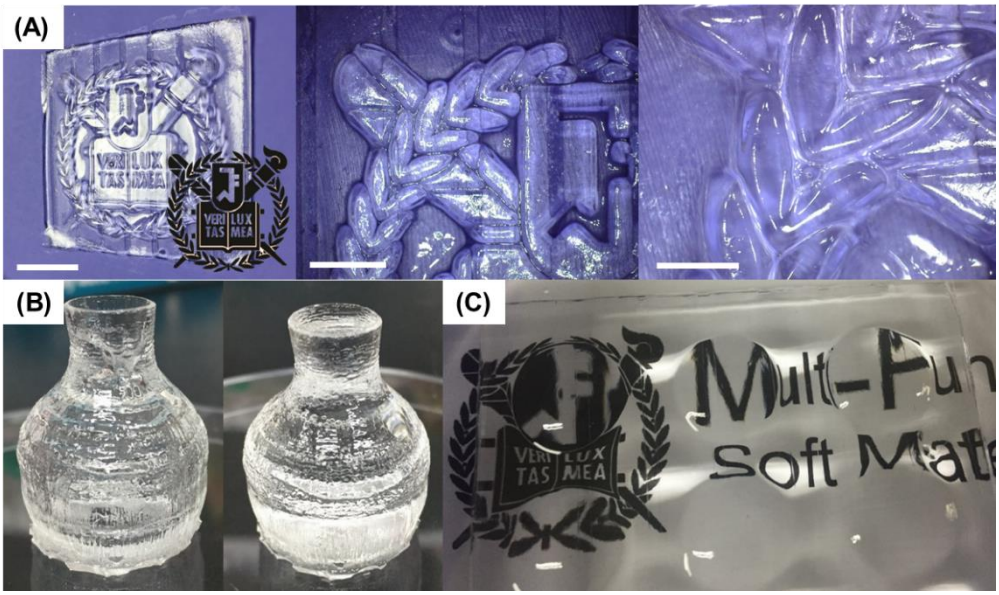


Figure 4.10 3D printed PAAm hydrogels (a) the emblem of Seoul National University (b) a vase (c) a lens array

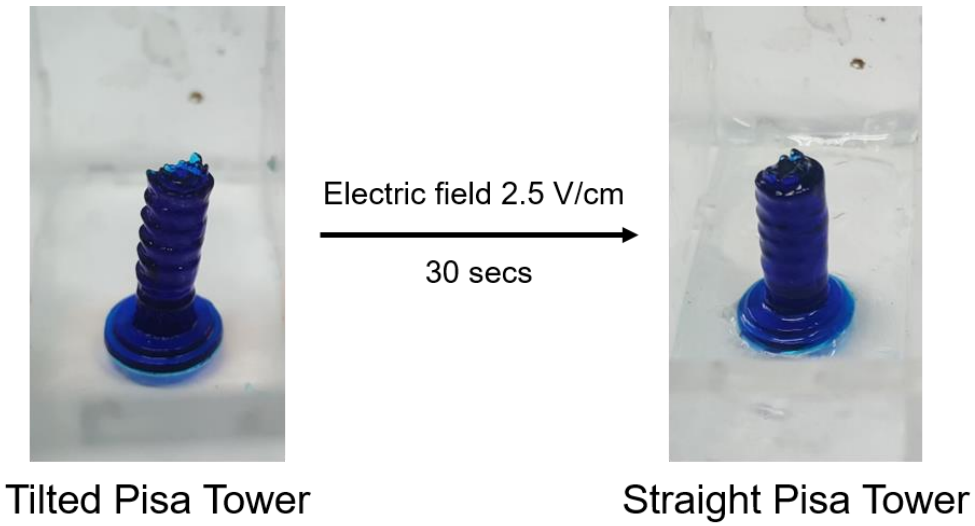


Figure 4.11 3D printed P(SPA-AAm) hydrogels and electroactivity

5. Conclusion

In this research, 3D printing of PSPA hydrogels was conducted using DLP apparatus. For the printing of hydrogels, non-commercial photoinitiator LAP and methacrylated hyaluronic acid as a crosslinker were synthesized. Addition of methacrylated hyaluronic acid solved the problem which is caused by fast polymerization of 3D printing method. Macromolecules with 2 MDa of molecular weight and multi-crosslinking points helped incompatibility of high elastic modulus with high stretchability. The macromolecules lengthened polymer chains by their own chain and increased crosslinking density by their multi legs. Insufficient mechanical properties of electroactive PSPA and PAETMAC hydrogels were enhanced by methacrylated hyaluronic acid. The PAAm hydrogel structures were printed by the DLP apparatus, and Pisa tower made of P(SPA-AAm) hydrogel was activated under an electric field.

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